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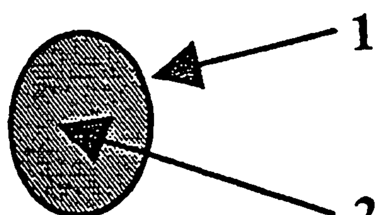
(54) Title: PIGMENT FEED



(57) Abstract: There is described an invention directed to a capsula with taste substances added and filled with a pigment solution to be fed to farmed fish, production of the capsula and use of the capsula.

PIGMENT F[illegible]

The invention relates to a capsule with taste substance filled with pigment, to color the fish meat of farmed fish, production and use of the capsule.

The slaughter quality of farmed fish is improved by starvation of the fish for a period of time before slaughtering. According to authority regulations, in order to reach superior quality, the starvation of the fish has to last at least until all feed residues in the fish are disappeared from the gastrointestinal system. The starvation period which is needed to attain this depends on the water temperature, but in practice this means about 14 days during winter time and 9 days in the summer. Starvation of farmed fish also has a positive effect on the market value, by reducing the fat content of the meat, making the meat more firm and improving the slaughter hygiene.

Salmon species are known by the distinctive red colour of the meat, which in fish farming depends on supply of pigments added in the feed. Pigments in the muscles are however continuously modified (oxidized/reduced) to colourless components. A continuously supply of pigments is therefore needed in order to maintain the red colour of the meat. According to current practice the pigment is supplied through the feed.

The pigmentation level of fish meat is one of the most important quality criterias for farmed salmon species. Low pigment level results in lower prices and negative reactions from the market, and is thus a considerable problem for the industry.

Thus the current situation is conflicting since maintenance of colour to satisfy the market requires feeding, while slaughter quality and reduced fat content, which also is important for the market value, require starvation which leads to a gradually loss of meat colour. In addition to loss of colour as a result of starvation, the pigment is poorly absorbed form regular feed (about 35 %) and constitutes about 25 % of the feed expenses. To reduce the conflict between feeding, starvation and its effect on market value, it is necessary to improve the absorption of pigments, during feeding and particularly in the starvation period before slaughtering by supplying pigment in a form which facilitates absorption.

The Norwegian patent, NO 302556, regards pigment feeding of fish and shellfish by a biodegradable polymeric basic matrix with alginate as a major component. According to this patent, there is however no information if the pigment is in a capsule or if the pigment feeding takes place during a starvation period in order to enhance the pigment content of the fish meat.

The object if the present invention is to provide alternative methods to pigment the meat of farmed fish. This object is obtained by the present invention characterized by the enclosed claims.

The present invention regards a capsule containing a physiologically acceptable pigment, with special characteristics regarding taste and solubility which makes it suited for feeding farmed fish, both in the feeding period and during starvation.

The invention regards further production of the capsule, and use of the capsule to increase the pigment content of the fish meat, both during regular feeding and during the starvation period before slaughtering.

The invention will in the following be described in more detail, with reference to figures and examples.

Figure 1 shows a section of a pigment capsule 1, filled with pigment dispersed in an oil with a suitable viscosity 2.

Figure 2 shows a spectrophotometric reading of a blood sample collected from a fish used in the experiment described in example 2; before the pigment capsule was given (0-sample) and 7 hours after feeding the capsule (7-hours). The ordinate shows the absorbance (ABS), while the abscissa indicates the wavelength (nm). The instrument base line is shown as a broken line.

Figure 3. Dose-response after administration of single doses of astaxanthin capsules in Arctic charr.

Figure 4. Accumulation of astaxanthin in blood of Arctic charr after single dose administration of astaxanthin capsules.

In order to reach the above mentioned intentions, a capsule in accordance with the present invention, must satisfy the following requirements;

Taste: The capsule must have a texture and a taste that is agreeable for this fish.

Degradability: To optimize absorption, the capsule must degrade quickly in the gastrointestinal system.

Absorption: The capsule must contain a optimum concentration of pigment, (soluted) in a suitable solvent, and the solution must have an adequate viscosity in order to ensure optimal absorption from the intestine.

Durability: The pigment has to keep its quality in the capsule.

Capsule weight: The capsule must sink in water.

Size: The capsule must be sized according to the fish it is intended for.

The present invention includes a capsule where taste substances are added to the capsule material, and where the capsule is filled with a physiological acceptable pigment mixed with an oil with a suitable viscosity.

Capsules are currently produced from different substances (gelatin, alginat etc.) for a variety of purposes. Existing capsules however need a modification in order to function as pigment capsule for fish. This modification mainly comprises producing a capsule with the right size and taste for the fish to eat voluntarily. Since such a capsule does not exist today, this will represent a new product. It is a well known fact that the appetite of farmed fish differs in relation to the food. According to this invention, feed extracts will be added to the capsule material in order to get the suitable taste for voluntary eating. In a different embodiment, amino acids produced from extracts of natural feed will be added. These taste-adding amino acids can for instance be produced from shrimp or squid.

The capsule decomposes in the stomach or in the intestine. As with mammals, fish have different pH in the stomach and intestine, with low pH in the stomach (pH 2-4) and higher pH in the intestine (pH 6.5-9). When for instance the pigment astaxanthin is in contact with acid, part of the pigment will be converted into colourless compounds. In other words; by giving the pigment by traditional methods through the feed, parts of the pigment will decompose already in the stomach, which most likely increases the need for more pigment in order to obtain adequately pigmented fish muscle. By using a capsule that decomposes in the intestinal pH range, one will obtain the requested pigmentation as with regular feeding, but with a far less amount of pigment. This represent a major economic advantage.

The capsule pigment is carotenoids, for instance selected from xanthofyll, such as astaxanthin, which is the one used currently to feed farmed salmon. Other useful pigments are cantaxanthin. The pigment is dissolved in water and mixed with an oil which optimizes absorption in the intestine. Absorption is regulated by the oil viscosity. By increasing the oil viscosity, the capsule time spent, in the intestine will increase and thereby the absorption of the pigment is enhanced.

According to the invention, the capsule will be used during normal feeding and instead of feed for salmon species during the starvation period, which is about 2 weeks before slaughtering. This is a novel feature, since pigments are currently not given during the starvation period. Since pigments are poorly absorbed from regular feed, the pigment capsule will be produced to maximize absorption. This is done by using an oil with the suitable viscosity, which however is not possible

regarding regular feed. This method will increase the pigment absorption, compared to the absorption from presently used fish feed. This will also make use of the capsule with regular feed during the feeding period economically profitable.

Production of the capsule will be in accordance with a well known technique. The material must be physiologically acceptable and must be produced either from gelatine or alginate. In order to attain the desired taste, the material will be added suitable extracts or amino acids. This will vary depending on the fish species. Further, the gelatineous material will be modified according to the desired localization of the degradation, in the stomach or in the intestine. The pigment will be dissolved in a solvent, such as for example water, and mixed with an oil with an optimized viscosity and filled on a capsule.

Examples

The objective with the examples is to show that a gelatine capsule, for instance filled with cod liver oil can degrade in the fish intestine (Example 1) and the pigment filled in the capsule is absorbed in the intestine and gives elevated levels in the blood Examples 2 and 3).

Material

Arctic charr (Salvelinus alpinus) (0,6 kg) was used as test fish in Examples 1 and 2. The capsule was a gelatine cod liver oil capsule which is commercially available.

Example 1. Degradation of a gelatine capsule

After anaesthetizing the Arctic charr, a gelatine capsule was pushed gently down the fish throat. After 2 hours, the fish was killed and the stomach examined. The result showed an almost complete degradation of the capsule. Since the capsule was degraded, the example demonstrates that the capsule content will be exposed for absorption.

Example 2. Absorption of the pigment from the intestine to the blood

A gelatine cod liver oil capsule described in Example 1, was used in this example. The results from Example 1 demonstrated that the fish used for testing was able to degrade this type of capsule. The source of the pigment was Lukanin Pink™ which contained 4 % astaxanthin. This was dissolved in water (1:6) and fish oil (5:6), and further injected in a gelatine capsule which could contain 0,3 ml of the solution. The proportion between Lukanin Pink and the solvent was such that the capsule (0,3 ml) contained 1 mg astaxanthin, which gave a concentration of 3,3 mg/ml.

Before the experiment started, the fish was starved to ensure that any change in pigment level in the blood plasma arose from the pigment capsule. Blood samples

from vena caudalis was taken before the pigment capsule was given to the fish. This sample represented the zero level and was termed the "0-sample". The blood samples were immediately centrifuged in 5 minutes at 1500 rpm. The blood plasma was subsequently pipetted over into small plastic tubes and stored in a freezer (-20°C)for further processing. Then the test fish was given a pigment capsule orally as described in Example 1. After 7 hours new blood samples (7-hours) where obtained from the fish as described for the 0-sample and treated as described above.

<u>Analysis</u>
1 ml blood plasma was used for analyzing. The plasma was transferred to centrifuge tubes, 1 ml metanol was added and the sample whirl mixed in 10 seconds. Chloroform (3 ml) was then added to the sample and the sample was whirl mixed for another 10 seconds. The sample was subsequently left for 10 minutes before centrifuged for 10 minutes at 3000 rpm. 2 ml of the chloroform phase was taken out for reading in the spectrophotometer. Astaxanthin has a maximum light absorption between 470 and 490 nm. The spectrophotometric reading was done in the Medical Research Laboratories at the University of Tromsø. The results are shown in figure 2.

Results and discussion
The aim of the above examples was to elucidate whether the test fish (Arctic charr) had the ability to degrade the gelatine capsule and if the pigment in the capsule could be absorbed from the intestine into the blood.

The results from Example 1 demonstrated clearly that the test fish could degrade the gelatine capsule in the stomach.

The results from Example 2 (figure 2) shows an elevated level of astaxanthin in blood plasma 7 hours after the orale intake, compared to the level in the blood samples taken before intake of the capsule. The sample taken after 7 hours, shows a marked peak at about 475 nm on the spectrophotometer curve, which is typical for astaxanthin. Analysis of the blood sample taken before the intake of the pigment capsule (0-sample), showed a considerable smaller peak in the same wavelength range, and shows that the 0-sample contained a minimum concentration of astaxanthin. This demonstrates that the capsule is dissolved in the test fish and the pigment is made available for absorption from the instestine into the blood.

<u>Example 3</u>. This example demonstrates that administration of capsules filled with astaxanthin to Arctic charr resulted in a dose-response blood concentration of the pigment and time related accumulation of the blood content of astaxanthin.

The aim of the present invention is to develop a capsule containing astaxanthin or a similar carotenoid for muscle pigmentation of fish such as salmonid sp. The product is primarily intended for use during starvation periods or in periods in periods in which the fish is not fed due to feed quota. No such product is on the market at present. Astaxanthin uptake and deposition is low. In small Atlantic salmon, (140-740 g) astaxanthin digestibilities of 46-59 % has been found, while 4 % of the ingested amount of astaxanthin was retained in the muscle (Bjerkeng and Berge, 2001. Comp. Biochem. Physiol 127B, 423-432). Low utilization is reported in the other relevant salmonid fishes. If the pigment capsule developed in this project is efficient, it represents a viable strategy to use this for pigmentation.

The present example regards investigating the potential to develop such a capsule based on the predetermined technological properties set for the product. We have therefore conducted three different trials in which we determine dose-response, accumulation of astaxanthin in the blood as a measure of bioavailability and finally accumulation of astaxanthin in the muscle after administrating astaxanthin-containing capsules. The first two trials are terminated, whereas samples from the muscle pigmenting trial now is being analyzed. Figure 3 shows the dose-response for the most promising pigment-capsule. The trial showed that in Arctic charr we have a positive dose-response up to 2 mg pr. fish. For fish of this size this corresponds to ca. 6 mg/kg body weight. The time-concentration-curve after single-dose administration of astaxanthin capsules showed increasing concentration for capsules 1 and 2 (series 2 and 3) up to 60 hours (trial 1), Figure 4. This is somewhat surprising because maximum blood concentration following single-dose administration of astaxanthin in Atlantic salmon and rainbow trout is reported to be 18-30 hours. The reason may be a low flow through the gastrointestinal tract in our experiments due to starvation. This indicates that the utilization of astaxanthin is high. This will be revealed when the data from the muscle pigmentation trial is examined.

CLAIMS

1. Capsula for feeding fish to maintain and/or increase the content of pigment in the fish meat, characterized in that it comprises a capsula material with taste substances added and filled with a pigment solution, wherein the capsula material which consists of gelatine or alginate is dissolved in stomach or intestine respectively, contains taste substances selected among extracts from the natural feed of the fish, wherein the taste substances can be selected among amino acids produced from extracts of the natural for the fish and that the pigment is mixed with oil.

2. Capsula according to claim 1, characterized in that the amino acid is produced from shrimp or octopus.

3. Capsula according to claim 1 or 2, characterized in that the pigment is selected from the group containing carotenoids, such as xhantofyll.

4. Capsula according to claim 3, characterized in that the xanthofyll is astaxanthin or cantaxanthin.

5. Capsula according to claim 4, characterized in that the xanthofyll is astaxanthin.

6. Capsula according to the claims 1-5, characterized in that the pigment is mixed with animal oil, preferably fish oil.

7. Capsula according to claim 6, characterized in that the oil has a viscosity giving increase detention period in the intestine.

8. Method to produce the capsula according to claim 1-7, characterized in that pigment dissolved in water is mixed with fish oil and the mixture is injected into a capsula produced of gelatine or alginate, whereafter the capsula is inserted in a bath wherein suitable taste substances are added, or sprayed with a solution of taste substances, and subsequently dried in the air.

9. Method according to claim 8, characterized in that the taste substances are extracts from the natural feed of the fish, preferably amino acids from shrimp.

10. Method according to claim 8-9, characterized in that the pigment is astaxanthin, preferably Lukanin Pink.

11. Use of the capsula according to claims 1-7, wherein the capsula is fed to farmed fish to maintain and/or increase to content of pigment in the fish meat.

12. Use according to claim 11, wherein the capsula is fed to farmed fish in the starvation period before slaughtering.

Fig. 1

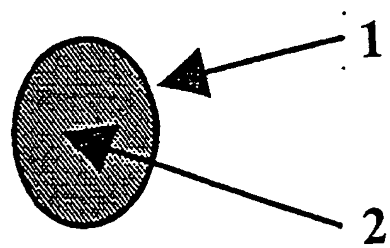


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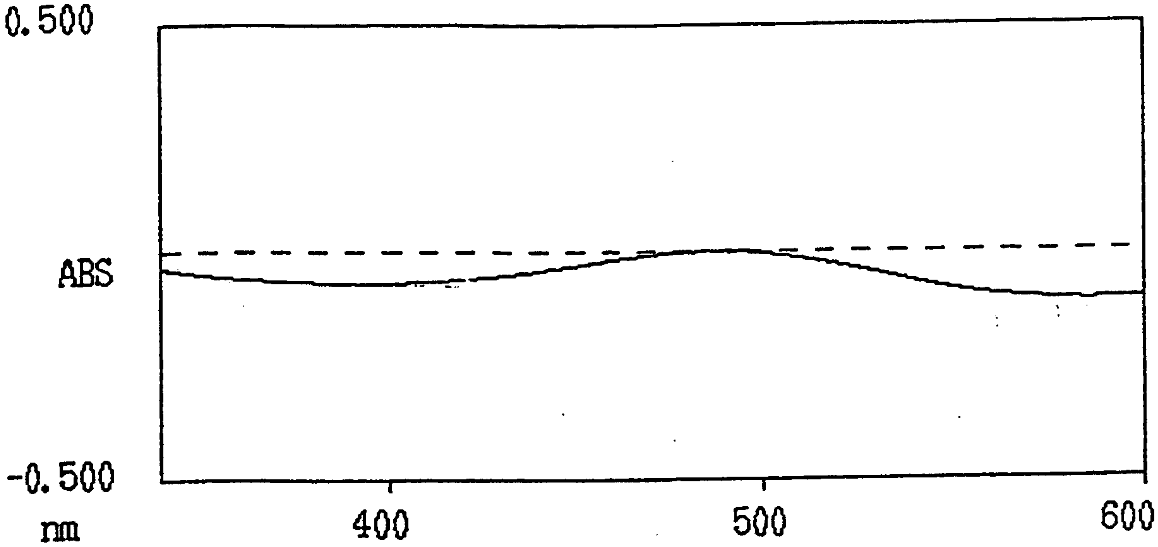
Fig. 2

0 - sample



7 - hours

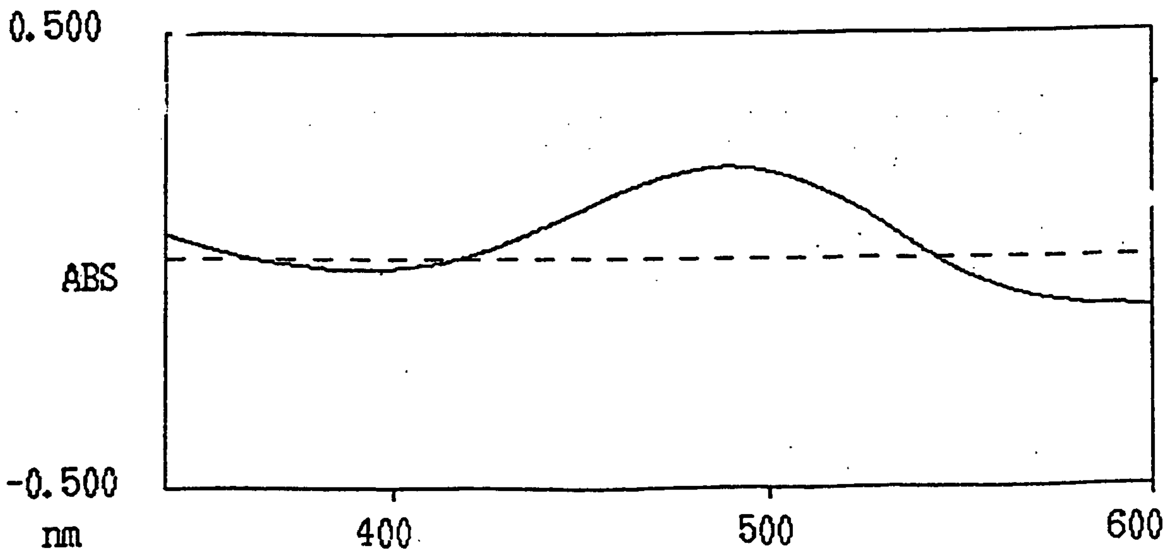


Fig. 3

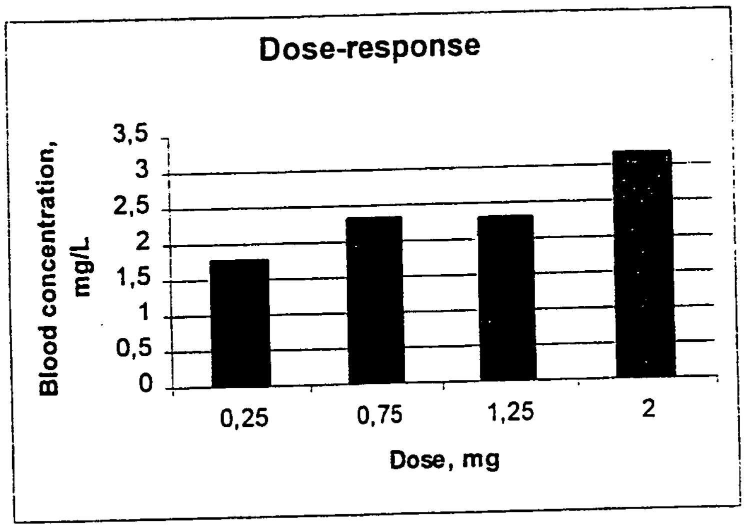


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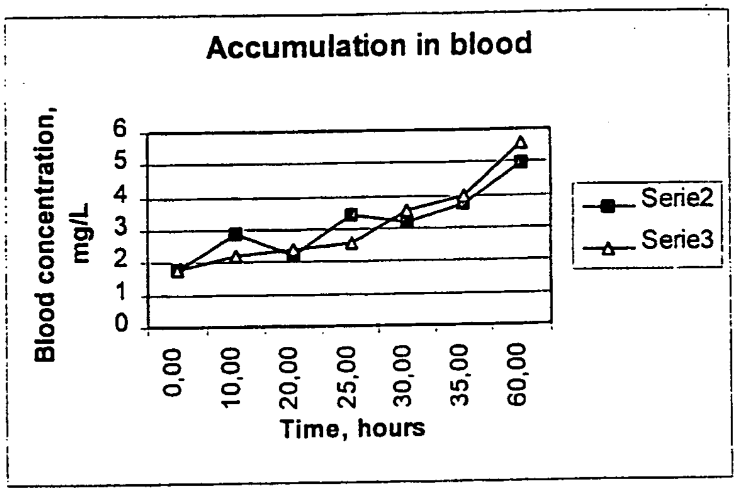
Fig. 4


Accumulation in blood
Blood concentration, mg/L
6
5
4
3
2
1
0
0,00
10,00
20,00
25,00
30,00
35,00
60,00
Time, hours
Serie2
Serie3